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Transinfections of the endosymbiont *Rickettsiella viridis* in different *Myzus persicae* (Hemiptera: Aphididae) clones show consistent deleterious effects and stable transmission

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Endosymbionts are widespread in insects, including aphids, and can have multiple effects on insect host fitness, suggesting potential applications for endosymbiont-related pest control. A transinfection of the endosymbiont *Rickettsiella viridis* into a line of the novel host *Myzus persicae* has previously shown large deleterious effects on aphid fitness and rapid spread in caged aphid populations under a cool environment. Because host clones can significantly influence endosymbiont effects and fitness-related traits more generally, it is important to test endosymbiont effects across a range of genotypic backgrounds. Here, we developed four *Rickettsiella* transinfected lines in different *M. persicae* clones via hemolymph microinjection, including clones with relatively high pesticide resistance. All four lines exhibited consistent fitness costs, reflected in reductions in both fecundity and longevity and reduced heat tolerance, although the magnitude of these effects varied among clones. The lines also resulted in stable and similar shifts in body color, with infected aphids being darker in color, although clonal effects were again observed. Vertical transmission was stable in all clones, and *Rickettsiella* infection was also shown to be transmitted horizontally between aphid pairs within Petri dishes in each clone. These results demonstrate consistent transmission and deleterious fitness effects of *Rickettsiella* transinfections, while also highlighting genetic background effects.

Keywords: Endosymbiont, *Myzus persicae*, aphid clone, transinfection, fitness effects, transmission

Introduction

In nature, maternally inherited bacterial symbionts are widespread, with nearly two-thirds of arthropods showing close associations with them (Moran et al. 2008). These symbionts often have profound effects on the ecology and evolution of their hosts (Oliver et al. 2010). Aphids (Hemiptera: Aphididae) are a key group of insects where the interaction between hosts and endosymbionts has been studied intensively. Aphid endosymbionts include the obligate *Buchnera aphidicola*, which provides essential amino acids (Douglas and Prosser 1992, Douglas 1998), as well as a variety of facultative endosymbionts that can result in fitness costs and/or benefits under certain environments (Chen et al. 2000, Leonardo and Muiru 2003, Heyworth and Ferrari 2015). The reliance of aphids on obligate endosymbionts, along with the influence of secondary endosymbionts, makes them a potential target for biological control (Berasategui et al. 2016, Gu et al. 2023).

With microinjection, it is possible to transfer endosymbionts between different aphid species, potentially generating aphid–endosymbiont combinations of interest for pest control (Gu et al. 2024). This follows previous work on pest transinfections, which have had a significant impact on the control of mosquitoes and their ability to transmit disease (Walker et al. 2011, Zheng et al. 2019). Transinfections with pest control potential have also been developed in agricultural pests, including fruit flies (Zabalou et al. 2004), planthoppers (Gong et al. 2020), and aphids (Gu et al. 2023). One important question in using endosymbionts is whether they have consistent and stable phenotypic effects across different genetic backgrounds and environments. In aphids, both genetic background (Lukasik et al. 2011, Wang et al. 2016) and environmental factors, such as plant hosts (Tsuchida et al. 2011, Wagner et al. 2015, Tougeron and Iltis 2022), can influence endosymbiont-induced host effects. These factors can affect the stability of endosymbionts within hosts and field populations, further influencing their capacity to provide novel pest control.

In earlier work on a *Rickettsiella viridis* transinfection in a line of *Myzus persicae,* Gu et al. (2023) showed that this endosymbiont had severe costs to fecundity and heat tolerance, but also that it could rapidly spread through caged aphid populations via horizontal (by plant tissues or physical contact) and vertical (from mother to offspring) transmission. The infection might be expected to suppress wild aphid populations by inducing deleterious effects once released repeatedly into the field, as supported by models (Slavenko et al. 2024), although this is likely to depend on host plant composition and other environmental conditions (Ross et al. 2024).

In the current study, we consider the stability of phenotypes induced by *Rickettsiella* among different clones of *M. persicae* found in Australia, where sexual reproduction is limited and several clonally reproducing clades exist (Thia et al. 2025), which can differ in pesticide resistance (Umina et al. 2022). We generated four *Rickettsiella*-infected *M. persicae* clones with different genotypes using hemolymph microinjection and then compared life history traits, heat tolerance, and horizontal transmission of different clones, with and without *Rickettsiella* infection. Our results showed that the infection altered aphid body color and exhibited consistent phenotypic costs in terms of fecundity, longevity, and heat tolerance, although there were quantitative differences in the costs incurred. Horizontal transmission efficiency also varied among clones, which may influence spread ability in populations (Slavenko et al. 2024). The consistent effects of *Rickettsiella* across *M. persicae* clones are important for future field releases, where there is potential for horizontal transmission across different clones (Gu et al. 2023).

Materials and Methods

Aphid Lines and Maintenance

As the donor species in the microinjection experiment, we used a single clone of *Acyrthosiphon pisum,* which was originally collected from Tintinara, South Australia (GPS: -35.83, 140.13) and carried both *Serratia symbiotica* and *Rickettsiella viridis*. We used four clones of *M. persicae* which were free of any naturally infected secondary endosymbionts: Clone 37, Clone 98, Clone 171, and Clone 188. Clone 37 was collected from Toongabbie, Victoria (GPS: -38.07, 146.62) in 2020 from *Brassica napus*. Clone 98 was originally collected from Kyabram, Victoria (GPS: -36.38, 145.03) in 2002 from *Capsicum annuum*. Clone 171 was collected from Osborne, Queensland (GPS: -19.71, 147.36) in 2020 from *Capsicum frutescens*. Clone 188 was provided by the Grains Innovation Park (Horsham, Victoria). Clone 171 is known to possess a wide range of insecticide resistances, including to organophosphates, pyrethroids, carbamates, neonicotinoids, sulfoximines, and tetronic and tetramic acid derivatives, and Clone 188 also has a number of resistances, including to organophosphates, pyrethroids and carbamates, while Clone 98 is susceptible to all insecticides (Umina et al. 2014, 2022, Kirkland et al. 2023). The insecticide resistance status of Clone 37 is unknown but the clone was chosen due to its ability to become infected via plant-mediated horizontal transmission in a previous study by Gu et al. (2023). *Myzus persicae* from each clone were genotyped at the outset of this study to confirm their clonal make-up. Five aphids from each clone were genotyped using ten previously described DNA microsatellite loci (Sloane et al. 2001, Umina et al. 2014). Aphids were also genotyped on multiple occasions during the study to ensure cultures remained uncontaminated (ie clones had not become contaminated with other aphid genotypes). All fitness and heat shock experiments were conducted using aphids from the same generation for all four clones (around G15 post-transinfection) and horizontal transmission experiments were done using aphids from G18.

All *M. persicae* clones were cultured on Petri dishes (60 mm × 15 mm) with bok choy (*Brassica rapa* subsp. Chinensis) leaf discs placed on a layer of 1% agar and *A. pisum* was cultured in a similar manner except lucerne (*Medicago sativa*, cv. Sequel) stems and trifoliate leaves were used as the plant host material. Aphids were maintained in a temperature-controlled room at 19 ± 1 °C with a 16:8 L:D photoperiod and transferred to new Petri dishes with fresh plant host material once a week. Plants were grown in a shadehouse with plant growth lights (40W Grow Saber LED 6500K, 1200 mm length) set to a 16:8 L:D photoperiod.

Endosymbiont Transfer Through Microinjection

We introduced *Rickettsiella* from *A. pisum* into the four aforementioned clones of *M. persicae* via microinjection. Three different development stages -early nymphs (3-d old), late nymphs (5-d old), and young adults (7-d old)—were established to examine the success rate of transinfection after microinjection. To transfer endosymbionts, hemolymph was withdrawn from donor aphids and immediately injected into the recipient aphids using a MINJ-1000 microinjection system (Tritech Research), as described previously (Gu et al. 2023, 2024). Thirty aphids (G0) per development stage were injected with hemolymph. Variable numbers of *A. pisum* were used as donors based on their body size, with one *A. pisum* adult providing a source for five *M. persicae* early nymphs, two *M. persicae* late nymphs or one *M. persicae* young adult. Injected aphids were maintained at 19 ± 1 °C in groups of ten individuals per Petri dish (60 mm × 15 mm) containing bok choy placed on 1% agar. We checked the survival after microinjection three times per week. After 10 d, surviving G0 aphids were placed individually in new Petri dishes. Once more than four nymphs (G1) were produced, injected G0 aphids were stored in 100% ethanol and frozen at –20 °C until being screened for *Rickettsiella* infection using qPCR (see 'Endosymbiont detection and quantification'). Only the nymphs from mothers with high *Rickettsiella* density (Cp < 25) were selected for the next generation. This process was repeated until a 100% infection frequency and high density were observed in G3. We maintained the *Rickettsiella* (+) lines as a mixed population from G4. Fifteen aphids at G4 were kept in a group and allowed to produce nymphs for 7 d. After obtaining enough nymphs for G5, all 15 individuals were stored and then tested for the presence of *Serratia*. Individuals from the *Rickettsiella* (+) lines were screened via qPCR routinely (every

2 to 3 generations) to ensure that the infection was maintained at a high frequency. All the *Rickettsiella* (+) lines maintained a stable infection for at least 10 generations post-injection before experiments began. Eight lines were used for all comparisons in the following experiments: four *Rickettsiella* (+) lines generated via microinjection and four naturally uninfected *Rickettsiella* (-) lines.

Effects of *Rickettsiella* Infection on Life History Traits

Rickettsiella (-) and *Rickettsiella* (+) *M. persicae* from each of the four clones were reared at a constant temperature of 19 ± 1 °C to assess the impact of different genetic backgrounds on phenotypic effects caused by *Rickettsiella*. Individual nymphs (< 24 h old) were transferred to Petri dishes (35 mm × 10 mm) containing bok choy placed on 1% agar. Forty replicates from each of the four *Rickettsiella* (+) and four uninfected *Rickettsiella* (-) lines were established and aphid survival checked daily until all individuals became adults. Developmental time was recorded based on the time that the aphid produced its first nymph. Aphids reaching adulthood were subsequently transferred to new Petri dishes containing bok choy placed on 1% agar twice a week. Fecundity was determined by counting the total number of nymphs produced during an individual's lifetime. The number of nymphs produced was counted three times per week until the adult died. Aphids that died before producing offspring were removed from the analysis. Forty replicates per infection status were set up for each aphid clone, with a total of 320 aphids were used to measure the fitness traits.

Body Color and Body Length

The effect of *Rickettsiella* on body color and body length were examined using age-matched aphids reared at a constant temperature of 19 ± 1 °C. Body length was measured by taking photos with a Leica MS5 microscope camera on 8-d-old aphids, and then analyzed in Image J with linear measurements taken from the front of the head to the rear of the abdomen (excluding the cauda). Both 8-d-old and 21-d-old aphids were used to examine body color. Living aphids were placed on filter paper inside a Petri dish and placed under a dissecting microscope (Leica MS5, 40 × magnification). Photographs of each aphid were captured using a Leica MS5 microscope camera. Between 20 and 40 individuals (replicates) from each of the four *Rickettsiella* (+) and four uninfected *Rickettsiella* (-) lines were measured, following the procedure in a previous study (Gu et al. 2023). Photographs were analyzed with ImageJ by selecting 150 × 150 pixel circles on the abdomen and obtained average Red Green Blue (RGB) values using the RGB measure plugin (https://imagej.net/ij/plugins/rgb-measure.html). RGB values were then converted to HSL (hue, saturation, lightness) in Microsoft Excel for aphid body color.

Heat Tolerance

Heat knockdown assays (Bak et al. 2020) were performed on 10-d-old aphids to assess the heat tolerance of *Rickettsiella* (+) and *Rickettsiella* (-) aphids from each clone. Sixty individuals (replicates) from each of the four *Rickettsiella* (+) and four uninfected *Rickettsiella* (-) lines were included in the experiment, which closely followed the methods of Bak et al. (2020). Aphids were placed individually into 5 ml glass vials and sealed with a plastic screw-top lid. Vials were then randomized in order, clipped to a plastic rack and fully submerged in a water tank set to a constant temperature of 41.5 °C. The time taken for each aphid to become incapacitated (no movement in 5s) was recorded as the knockdown time.

Horizontal Transmission in *Myzus persicae*

We tested horizontal transmission by mixing a single 1-d-old *Rickettsiella* (+) and a single *Rickettsiella* (-) aphid at the same age together and placing them on a Petri dish (35 mm × 10 mm) with a bok choy leaf disc placed on agar (Gu et al. 2023). Aphids mixed in the same Petri dish were from the same clone, with each set up replicated 60 times. Additionally, five replicates were established with either two *Rickettsiella* (+) individuals or two *Rickettsiella* (-) individuals in the same Petri dish, which acted as positive or negative controls, respectively. This experiment was undertaken for the four *Rickettsiella* (+) and four *Rickettsiella* (-) lines maintained in a temperature-controlled room at 19 ± 1 °C with a 16:8 L:D photoperiod. After 12 d, aphid pairs were stored in 100% ethanol and frozen at −20 °C until being screened for *Rickettsiella* infection using qPCR. Replicate dishes where one or both aphids died were excluded from the analysis.

Endosymbiont Detection and Quantification

We used qPCR assays to confirm the infection status and density of *Rickettsiella, Serratia,* and *Buchnera*. DNA was extracted using 250 µL of 5% Chelex 100 resin (Bio-Rad Laboratories), and endosymbiont detection and quantification was performed using a LightCycler 480, as described in Lee et al. (2012). In our previous study (Gu et al. 2023), we showed the abundance of the symbionts *Rickettsiella* and *Buchnera* was stable across generations at 19 °C, the temperature we also used for experiments in this study. In the current work, we only examined *Buchnera* density at G15 which was the same generation as used for the fitness and heat shock experiments. Each run included several positive controls: DNA from three *Rickettsiella* (+) and *Rickettsiella* (-) *M. persicae* of known infection status, as well as original donor aphids (*A. pisum*) carrying both *Rickettsiella* and *Serratia*. Negative controls were also included, which consisted of primers and water only. Four primer sets (Supplementary Table S1) were used to amplify markers specific to *M. persicae* (actin, *Rickettsiella, Serratia,* and *Buchnera*). The relative densities of *Rickettsiella, Serratia,* and *Buchnera* were determined by subtracting the Cp value of each endosymbiont-specific marker from the Cp value of the aphid-actin-specific marker. The differences in Cp values were averaged across 2 to 3 replicate runs and then transformed by $2^{\Delta Cp}$. These transformed values are displayed on a log scale (Log_{10}) in the figures.

Statistical Analysis

All statistical analyses were performed in SPSS Statistics 29 for Mac. General linear models (GLMs) were used to test the effects of clonal type, *Rickettsiella* infection, and their interactions on body color, fitness traits, and heat tolerance. Development time data were log transformed to improve the normality of residuals. Cox regression was applied to assess the impact of *Rickettsiella* infection on aphid longevity. Tukey's post-hoc tests or *t*-tests/Kruskall–Wallis tests with a Bonferroni correction for multiple comparisons were used to assess differences between aphid line pairs.

Results

Rickettsiella *Transinfection and Survival Comparisons*

Rickettsiella was successfully transferred from *A. pisum* to all four *M. persicae* clones via hemolymph microinjection. After microinjection, the highest mortality was observed at 2 d post-injection across

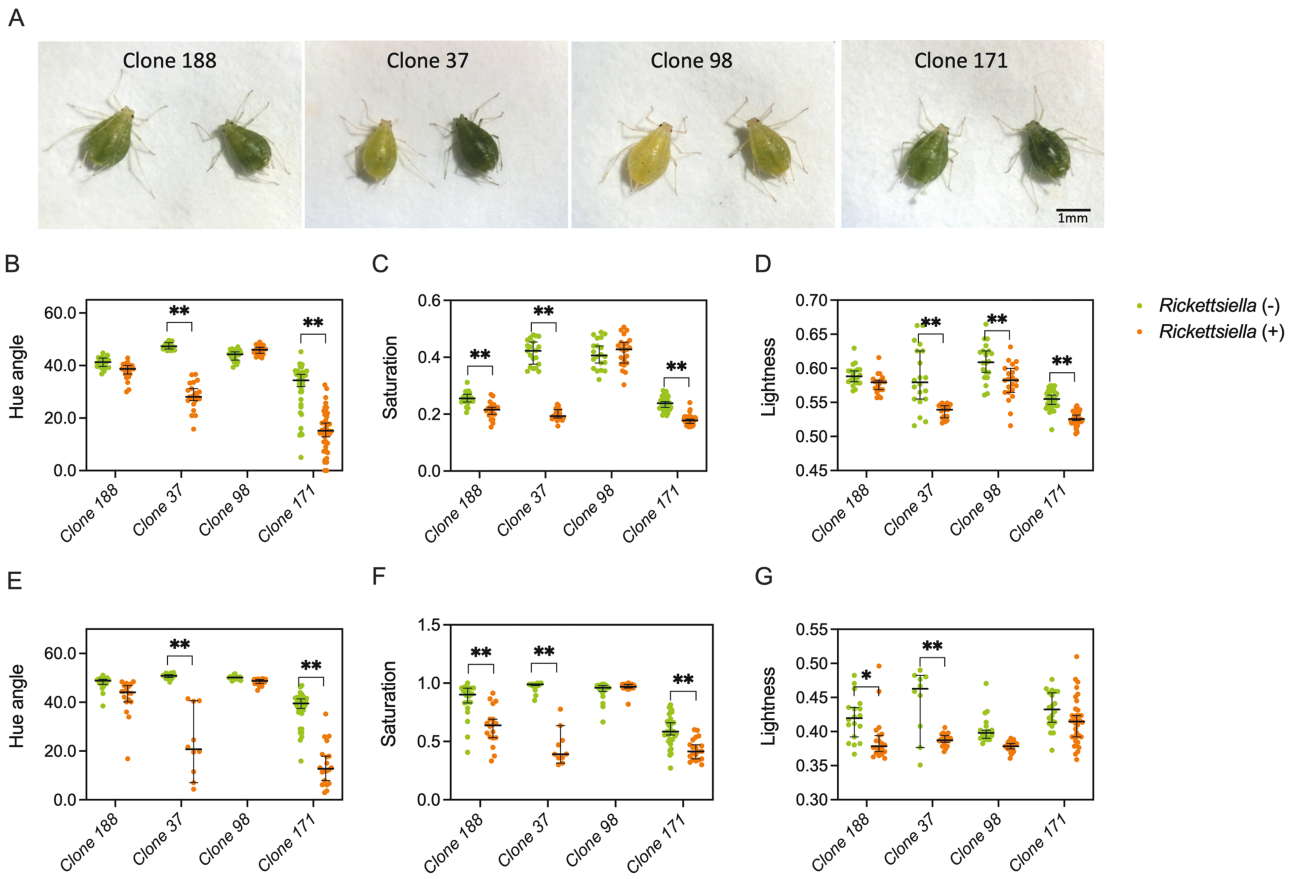

Fig. 1. Effects of *Rickettsiella* on body color in *M. persicae* in young (8-d old, B-D) and older (21-d old, E-G) adults from four clones. Body color of four *M. persicae* clones are shown in young adults (A), with *Rickettsiella* (-) individuals on the left and *Rickettsiella* (+) individuals on the right. Body color in young and older adults was split into three components: hue (B, E), saturation (C, F) and lightness (D, G). Data were analyzed with ImageJ by measuring individual body color components twice and averaging the two measurements. Dots represent data for individual aphids, while vertical lines and error bars are medians and 95% confidence intervals, respectively. Significant differences are marked with '*' and '**' above the dots, representing $P < 0.05$ and $P < 0.01$ by independent t-tests between *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae*, respectively.

all age groups. Survival rates after 10 d were similar for 3- and 5-d-old nymphs, but much lower for 7-d-old aphids (Supplementary Fig. S1). *Rickettsiella* was detected in all four clones at G0, with a very high infection frequency (Supplementary Fig. S2A–C). Transmission success varied among all four clones at different ages, with 7-d-old aphids exhibiting higher transmission success and stable infection density compared with the other age groups (Supplementary Fig. S2D–F) from G0 to G1. Both 3-d and 7-d-old aphids had higher transmission rates than 5-d-old individuals, but the latter group showed higher densities at G2 (Supplementary Fig. S2H–J). Given the variation in density and transmission rate, we performed a selection process in the first two generations following transinfection. From G3 onwards, all transinfected lines remained stable with a 100% transmission rate and consistently high *Rickettsiella* density. The endosymbiont *Serratia* was lost in all *M. persicae* clones after *Rickettsiella* reached a stable infection (*Serratia* infection at G4: 0 out of 15 aphids positive in all four clones), consistent with previous observations (Gu et al. 2023).

Rickettsiella *Infection Modifies Body Color to a Different Extent Among Aphid Clones*

One of the most prominent changes following *Rickettsiella* transinfection was a shift in body color, with *Rickettsiella* (+) aphids displaying a darker green compared with *Rickettsiella* (-) aphids. Infection status significantly affected all three color components in both 8-d-old (GLM: Hue: $F_{1,195} = 114.639$, $P < 0.001$, Fig. 1A; Saturation: $F_{1,196} = 259.294$, $P < 0.001$, Fig. 1B; Lightness: $F_{1,196} = 91.688$, $P < 0.001$, Fig. 1C) and 21-d-old aphids (GLM: Hue: $F_{1,157} = 213.093$, $P < 0.001$, Fig. 1D; Saturation: $F_{1,157} = 137.549$, $P < 0.001$, Fig. 1E; Lightness: $F_{1,158} = 49.061$, $P < 0.001$, Fig. 1F). Additionally, clonal type influenced body color in both in 8-d old (GLM: Hue: $F_{3,195} = 152.979$, $P < 0.001$, Fig. 1A; Saturation: $F_{3,196} = 406.299$, $P < 0.001$, Fig. 1B; Lightness: $F_{3,196} = 74.370$, $P < 0.001$, Fig. 1C) and 21-d-old aphids (GLM: Hue: $F_{3,157} = 130.682$, $P < 0.001$, Fig. 1D; Saturation: $F_{3,157} = 114.720$, $P < 0.001$, Fig. 1E; Lightness: $F_{3,158} = 11.422$, $P < 0.001$). The degree of body color change due to *Rickettsiella* infection varied among clones, with significant interactions between clone and infection found in 8-d-old (GLM: Hue: $F_{3,195} = 35.250$, $P < 0.001$, Fig. 1A; Saturation: $F_{3,196} = 93.312$, $P < 0.001$, Fig. 1B; Lightness: $F_{3,196} = 5.305$, $P = 0.002$, Fig. 1C) and 21-d-old aphids (GLM: Hue: $F_{3,157} = 40.255$, $P < 0.001$, Fig. 1D; Saturation: $F_{3,157} = 33.187$, $P < 0.001$, Fig. 1E; Lightness: $F_{3,158} = 3.014$, $P = 0.032$).

In 8-d-old aphids, significant differences in all body color traits were observed in Clone 37 and Clone 171 (Fig. 1, all $P < 0.001$). Significant changes were also found in Clone 188 for saturation ($P < 0.001$) and in Clone 98 for lightness ($P < 0.001$). However, in

21-d-old aphids, the color differences became less pronounced, with only Clone 37 showing significant changes across all color traits (all $P < 0.001$), while Clone 98 exhibited no significant changes (all $P > 0.100$).

Rickettsiella *Infection Reduces Aphid Fitness as Measured by Development Time, Fecundity, and Longevity*

Life history parameters measured showed that *Rickettsiella* infection resulted in a slower development time (GLM: $F_{1,302} = 6.275$, $P = 0.013$, Fig. 2A), defined as the time from the nymph stage to reproductive maturity. Development time varied among clones ($F_{3,302} = 33.436$, $P < 0.001$) and infection status ($F_{1,302} = 6.275$, $P = 0.013$), with Clone 37 showing slower development. There was also a significant interaction between infection and clone for development time ($F_{3,302} = 3.815$, $P = 0.001$).

Fecundity was significantly reduced by *Rickettsiella* infection ($F_{1,302} = 162.531$, $P < 0.001$), with a 20% to 50% decrease in total offspring produced in *Rickettsiella* (+) aphids compared with *Rickettsiella* (-) aphids (Fig. 2B). Fecundity was also influenced by clone ($F_{3,302} = 50.864$, $P < 0.001$), and the interaction between clone and infection was significant ($F_{3,302} = 9.346$, $P < 0.001$), with smaller impact of the infection on fecundity in Clone 98.

Rickettsiella infection also reduced longevity (Cox regression: $\chi^2 = 125.068$, d.f. = 1, $P < 0.001$, Fig. 2C), with the effects influenced by clone ($\chi^2 = 50.864$, d.f. = 3, $P < 0.001$).

Body length (Fig. 2D) was not affected by *Rickettsiella* infection status ($F_{1,191} = 0.287$, $P = 0.592$) but did vary among clones ($F_{3,191} = 35.680$, $P < 0.001$). Additionally, there was a significant interaction between clone and infection ($F_{3,191} = 4.647$, $P = 0.004$), with the largest effect observed in Clone 188.

Rickettsiella *Infection Does Not Influence the Density of the Primary Endosymbiont Buchnera*

We measured the densities of *Buchnera* and *Rickettsiella* in all four clones (Fig. 3) at G15, the same generation as used for the fitness and heat shock experiments. *Rickettsiella* infection had no effect on *Buchnera* density (GLM: $F_{1,166} = 0.216$, $P = 0.643$, Fig. 3A) and the density was similar among clones ($F_{3,166} = 0.687$, $P = 0.561$), with no significant interaction between clone and infection status ($F_{3,166} =$

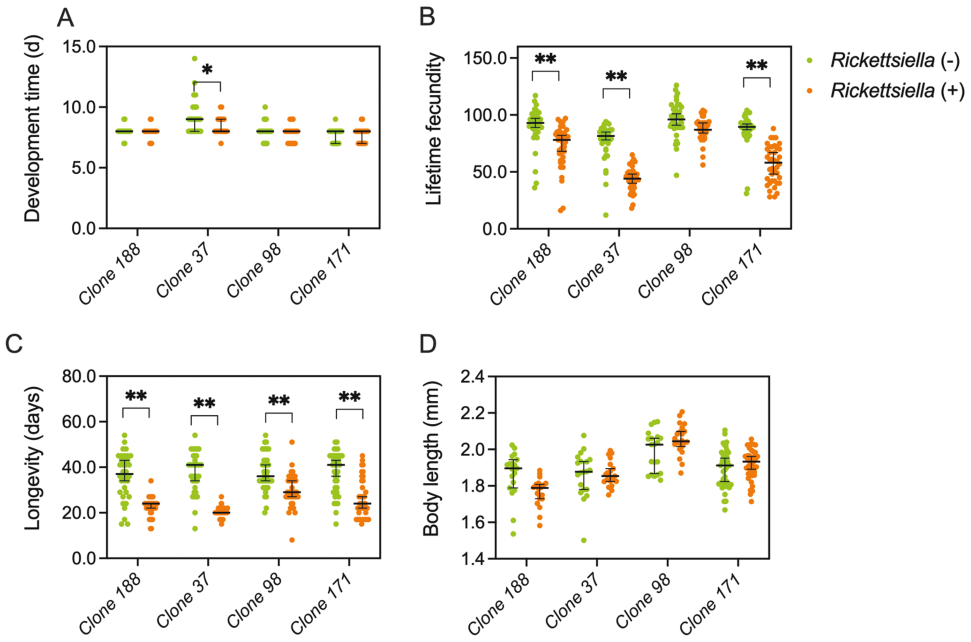

Fig. 2. Effects of *Rickettsiella* on life history traits in four clones of *M. persicae*. Development time (A), lifetime fecundity (B), longevity (C) and body length (D) were measured in *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae*. Dots represent data for individual aphids, while vertical lines and error bars are medians and 95% confidence intervals, respectively. Significant differences are marked with '*' and '**' above the dots, representing $P < 0.05$ and $P < 0.01$ by Kruskal-Wallis tests or independent t-tests between *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae*, respectively.

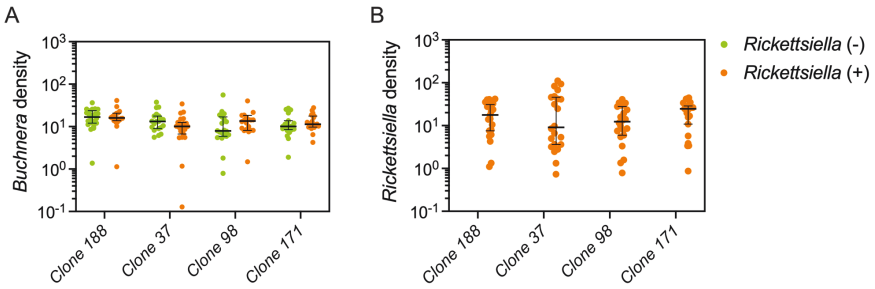

Fig. 3. Relative densities of *Buchnera* (A) and *Rickettsiella* (B) in *M. persicae* adults from four clones. Endosymbiont densities were measured when aphids were 8 d old. Endosymbiont densities were calculated relative to the actin marker and transformed by $2^{-\Delta Cp}$. Dots represent data for 20 to 25 individual aphids, while vertical lines and error bars are medians and 95% confidence intervals, respectively.

0.223, $P = 0.880$). Additionally, the density of *Rickettsiella* was not influenced by clone type ($F_{3,83} = 0.517$, $P = 0.672$, Fig. 3B).

Rickettsiella *Infection Reduces Aphid Heat Tolerance*

In a previous study, we found that *Rickettsiella* infection in Clone 188 decreased heat tolerance, as measured by CTmax and knockdown time (Gu et al. 2023). Here, we observed a similar effect related to infection status (GLM: $F_{1,462} = 34.577$, $P < 0.001$, Fig. 4). Overall, *Rickettsiella* (+) aphids had a shorter knockdown time compared with *Rickettsiella* (-) aphids. Knockdown time also varied among *M. persicae* clones ($F_{3,462} = 66.193$, $P < 0.001$), with Clone 188 and Clone 98 exhibiting a higher heat tolerance than Clone 37 and Clone 171 regardless of infection status. There was a significant interaction between clone and infection ($F_{3,462} = 11.197$, $P < 0.001$), with differences between *Rickettsiella* (+) and *Rickettsiella* (-) aphids only being significant in certain clones, as indicated by pairwise comparisons (Fig. 4).

Horizontal Transmission of Rickettsiella

All four *M. persicae* clones showed evidence of horizontal transmission between aphid pairs within Petri dishes, with > 11% of *Rickettsiella* (–) aphids testing positive for *Rickettsiella* in each of the four clones (Table 1: 'Frequency in recipient aphid' column). However, the densities were typically much lower in formerly uninfected *Rickettsiella* (-) aphids compared with *Rickettsiella* (+) aphids (Table 1: 'Median density in recipient aphid' column). The transmission rate varied between clones, with the highest transmission rate observed in Clone 37 followed by Clone 188 and Clone 171. The lowest transmission rate was observed in Clone 98. Cp values for the horizontal transmitted individuals which was *Rickettsiella* (-) tended to be much lower than in infected clones *Rickettsiella* (+) used in the transfers (with a mean of 18.92, range: 16.18 to 22.67, $n = 215$).

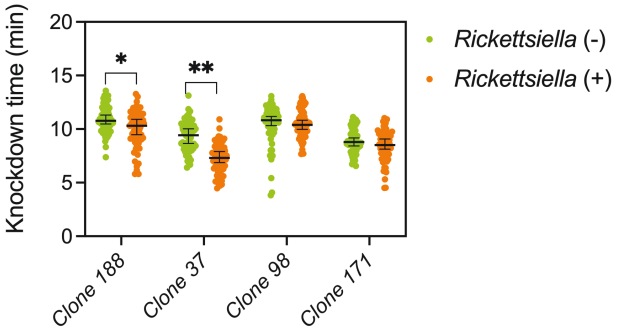

Fig. 4. Effects of *Rickettsiella* infection on heat tolerance in four clones of *M. persicae*. Heat knockdown town was tested at a constant 41.5 °C across all *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae* lines. Dots represent data from 60 individual aphids, while vertical lines and error bars are medians and 95% confidence intervals, respectively. Significant differences are marked with '*' and '**' above the dots, representing $P < 0.05$ and $P < 0.01$ by independent t-tests between *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae*, respectively.

Discussion

Here, we describe the interspecific transfer of *Rickettsiella viridis* and its phenotypic effects across different *M. persicae* clones. The *Rickettsiella* infection was stable in all four aphid clones over more than 20 generations, with no transmission leakage detected. But the other endosymbiont, *Serratia*, transferred at the same time, did not persist in any clone when tested at G4. The failure to transfer *Serratia* might reflect genomic divergence of the endosymbiont across hosts or perhaps interactions between *Rickettsiella* and *Buchnera* or the host genome (eg Rossi et al. (2015)). We note the failure of interspecific *Serratia* transfers into other aphid species from the same donor species (Tsuchida et al. 2006, Gu et al. 2024). *Rickettsiella* infection resulted in a shift in body color, significant fitness costs in terms of fecundity and longevity, and reduced heat tolerance. Interestingly, the magnitude of these effects differed among clones, and in Clone 37 in particular there were significant differences in all parameters between *Rickettsiella* (+) and *Rickettsiella* (-) aphids. The infection was horizontally transmitted between aphid pairs in Petri dishes, with varying transmission rates across clones. Vertical transmission remained stable in all clones, as indicated by the persistence of the infection over more than 20 generations. Overall, these results demonstrate the stability of *Rickettsiella*-induced phenotypes across clones, while also highlighting clone-specific differences.

We observed clear differences in life history traits between *Rickettsiella* (-) and *Rickettsiella* (+) *M. persicae*. *Rickettsiella* (+) aphids showed reductions in fecundity and longevity and exhibited a darker body color, consistent with our previous research (Gu et al. 2023) and also with some findings in its native host *A. pisum* (Tsuchida et al. 2010, 2014). Deleterious fitness costs may be due to *Rickettsiella* expressing pathogenic traits in insects generally (Bouchon et al. 2012). *Rickettsiella* may also induce cytoplasmic incompatibility, at least in spiders (Rosenwald et al. 2020). The

Table 1. Horizontal transmission rates of *Rickettsiella* among paired *M. persicae*. *Rickettsiella* infection status was determined using qPCR 12 d after aphids were placed on leaf discs within Petri dishes. Note: *Rickettsiella* (+) / *Rickettsiella* (+) and *Rickettsiella* (-) / *Rickettsiella* (-) represent positive and negative controls.

	Clone 188		Clone 37		Clone 98		Clone 171	
Aphid pairs	Frequency in recipient aphid	Median density in recipient aphid (range)	Frequency in recipient aphid	Median density in recipient aphid (range)	Frequency in recipient aphid	Median density in recipient aphid (range)	Frequency in recipient aphid	Median density in recipient aphid (range)
Rickettsiella (+) / *Rickettsiella* (-)	13/41 (31.7%)	37.00 (28.14 to 40.00, n = 41)	12/30 (40.0%)	32.07 (26.27 to 36.98, n = 30)	6/54 (11.1%)	32.27 (29.50 to 37.27, n = 54)	14/50 (28.0%)	38.08 (34.80 to 40.00, n = 50)
Rickettsiella (+) / *Rickettsiella* (+)	5/5	18.75 (17.92 to 18.93, n = 10)	5/5	18.26 (16.90 to 18.85, n = 10)	5/5	18.66 (17.57 to 19.50, n = 10)	5/5	18.81 (17.96 to 19.36, n = 10)
Rickettsiella (-) / *Rickettsiella* (-)	0/5	--	0/5	--	0/5	--	0/5	--

--: Not considered.

phenotypic effects identified in our study varied among clones, with significant interactions between clone and infection status. While the body color shift was evident across an aphid's lifetime (Fig. 1), the darker color of *Rickettsiella*-infected aphids was more pronounced in Clone 37 and Clone 171 compared with Clone 98. Body color therefore seems to be influenced by clonal genotype, infection status, aphid age, and interaction effects. A clonal effect on phenotypic effects of the infection was also evident in fitness traits, where infected Clone 37 and Clone 171 aphids had a larger reduction in fecundity and longevity compared with the effects of *Rickettsiella* infection in the other two clones. Although fitness traits varied among clones, the densities of *Buchnera* and *Rickettsiella* were similar across all four clones examined in this study. Thus, endosymbiont density does not appear to be directly related to the effects of the infection or clonal interactions. Interactions between the densities of co-existing endosymbionts have previously been noted for the native *Rickettsiella* infection in *A. pisum*, particularly with respect to the density of *Hamiltonella defensa* (Leclair et al. 2017). In the absence of an interaction between the density of *Rickettsiella* and the primary endosymbiont *Buchnera,* vertical transmission of *Rickettsiella* may be stable given the absence of selection for *Buchnera* to increase to a higher density (which could lead to higher host fitness).

Horizontal transmission can significantly influence the spread of endosymbiont infections within asexual aphid populations (Gu et al. 2023). We observed horizontal transmission in all four clones, with Clone 37 showing the highest rate (40%) and Clone 98 the lowest (11%). The low transmission rate in Clone 98 may be linked to a higher rate of alate production in this clone (unpublished data), with 42 out of 108 (39%) adults developing into alates, compared with a much lower percentage in the other clones (eg 5% in clone 171 based on 100 aphids). In *M. persicae*, alates probe less frequently than apterous individuals (Boquel et al. 2011), which may limit their ability to generate horizontal transmission of *Rickettsiella* through plant tissue when feeding. While we only tested transmission between infected and uninfected individuals of the same clone, it would also be valuable to test horizontal transmission rates between different clones. Additionally, testing transmission through different environments and host plants (Ross et al. 2024) would help better assess the range of conditions these aphids might encounter in the field.

Heat tolerance is an essential trait for both aphids and endosymbionts, the latter of which may be particularly vulnerable to high temperatures (Zhang et al. 2019). In any field application, variable temperatures in different regions and seasons may have a large impact on the successful establishment of aphid transinfection lines (Slavenko et al. 2024) which has been documented for the persistence of *Wolbachia* infected mosquitos following release (Ross et al. 2020, Caragata 2023, Vásquez et al. 2024). We previously observed a decrease in heat tolerance in *M. persicae* associated with *Rickettsiella,* as indicated by a reduction in knockdown time (Gu et al., 2023). A similar effect on heat tolerance was found in this study, although the effects varied among clones, which could influence the spread of the infection in specific clones. Clones with higher heat tolerance may spread faster and farther than others (Hall 1992, Barro et al. 1994, Oberle et al. 2010). Our previous study on a single clone suggested that *Rickettsiella* can spread effectively under cooler conditions but not under warm conditions (Gu et al. 2023), and spread might be further curtailed by brief periods of high temperatures, particularly if *Rickettsiella* is confined to a clone with inherently low heat tolerance. Clonal type should be carefully considered before any field releases of *Rickettsiella*-transinfected aphids are made. For example, Clones 98 and 188 might be preferable for releases under warmer conditions, such as in greenhouses.

It is important to note that we have so far tested only one strain of *Rickettsiella,* and other strains may exhibit different properties on host aphids when transinfected. *Rickettsiella* shows low genomic diversity across hosts in nature (Guyomar et al. 2018, Nikoh et al. 2018), in contrast to some other secondary endosymbionts, such as *Hamitonella defensa* and *Regiella insecticola* (Guyomar et al. 2018). Nevertheless, it will be important for future research to explore other strains of *Rickettsiella*, particularly those sourced from warmer regions.

In summary, our study highlights the stable and relatively consistent deleterious effects associated with *Rickettsiella* in multiple *M. persicae* clones, demonstrating its potential as a tool for aphid management in the field (c. f. Slavenko et al. 2024). While the infection consistently induced fitness costs and altered key traits such as body color and heat tolerance, the magnitude of these effects varied among clones. Other work indicates that *Rickettsiella* does not affect insecticide tolerance (Dorai et al. 2024) or provide protection against entomopathogenic fungi (Arinanto et al. 2024) or parasitoids (Soleimannejad et al. 2023) when present in *M. persicae*. This means current chemical control and biological control options will still be available if *Rickettsiella*-infected aphids are released into a population. Nevertheless, the current results together with previous studies on endosymbiont transfers to different aphid clones (Lukasik et al. 2013, Niepoth et al. 2018) underscore the importance of considering clonal differences when evaluating the ecological impact and potential applications of *Rickettsiella* in pest control. More work is needed to understand clonal differences of endosymbiont effects at the mechanistic level. Further research is also needed to explore the broader ecological implications of *Rickettsiella* presence such as possible trade-offs between the deleterious effects of *Rickettsiella* and potential benefits under field conditions including predation. This will allow the risks and opportunities of using this endosymbiont in agricultural applications to be evaluated.

Supplementary material

Supplementary material is available at *Journal of Economic Entomology* online.

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